Animal Models of Barrett's Esophagus and Esophageal Adenocarcinoma–Past, Present, and Future

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Abstract

Esophageal adenocarcinoma is the fastest rising cancer in the United States. It develops from long-standing gastroesophageal reflux disease which affects >20% of the general population. It carries a very poor prognosis with 5-year survival <20%. The disease is known to sequentially progress from reflux esophagitis to a metaplastic precursor, Barrett's esophagus and then onto dysplasia and esophageal adenocarcinoma. However, only few patients with reflux develop Barrett's esophagus and only a minority of these turn malignant. The reason for this heterogeneity in clinical progression is unknown. To improve patient management, molecular changes which facilitate disease progression must be identified. Animal models can provide a comprehensive functional and anatomic platform for such a study. Rats and mice have been the most widely studied but disease homology with humans has been questioned. No animal model naturally simulates the inflammation to adenocarcinoma progression as in humans, with all models requiring surgical bypass or destruction of existing antireflux mechanisms. Valuable properties of individual models could be utilized to holistically evaluate disease progression. In this review paper, we critically examined the current animal models of Barrett's esophagus, their differences and homologies with human disease and how they have shaped our current understanding of Barrett's carcinogenesis. Clin Trans Sci 2015; Volume 8: 841–847

Keywords: Barrett's esophagus, esophageal adenocarcinoma, animal models, surgical model, genetic model

Introduction

Adenocarcinoma of the lower esophagus, a relatively rare cancer, has increased sixfold over the last two decades and continues to escalate at a pace faster than any other solid cancer in the western world.[1] Despite advances in multimodal therapy, esophageal adenocarcinoma (EAC) portends an extremely poor prognosis with 5-year survival of <20%.[2] EAC is believed to develop in an orderly manner through a metaplasia-dysplasia-carcinoma sequence. Currently, it is understood that due to long-standing pathologic exposure to gastroesophageal refluxate, the normal stratified squamous mucosal cells transform to columnar mucosal cells with intestinal features (Barrett's esophagus; BE) both at the molecular and structural level. However, only 5–10% of patients with GERD develop BE[3] and <5% of BE progress to EAC.[4] Figuring out which patients would progress and which wouldn't is a major clinical dilemma. Most EAC patients are diagnosed without prior diagnosis of BE and many even without any prior symptoms related to GERD.[4] For this very reason, screening and surveillance efforts have also been rendered unsuccessful in significantly altering patient management. For patients detected even at the nondysplastic BE stage, acid suppression or ablative procedures do not guarantee regression or freedom form recurrence. At present, we are at a rather disturbing clinical standstill.

To bring any effective change in the current management of EAC, we must understand the underlying mechanistic aspects of its natural history. Elementary aspects such as the cellular source of origin, the carcinogenic component of the refluxate, the role of metaplastic change in priming for further progression and the essential mutations needed for carcinogenesis are not fully known. The low incidence of EAC and the silent long course of its developmental progression makes it hard to investigate its natural history in humans. Epidemiological associations are inherently insufficient in interpreting causality. Although tissue culture models and organotypic models have been attempted, they cannot represent the genetic diversity, clonal dynamics, and stromal and host–immune interaction involved in clinical neoplastic progression.[5] Animal models can, however, provide such a holistic platform. Animals with a structurally similar foregut which either spontaneously or with deliberate surgical or genetic alteration, show similar pathogenic events, can be a useful resource. A translatable and reproducible model once created, may then be manipulated for individual risk factors in a controlled setting to precisely understand their role in pathogenesis. Hypothesis-independent genome-wide analysis of longitudinally sampled precancerous tissue from these animals can help delineate the molecular succession preceding cancer development.

Encouragingly, many such attempts at developing animal models for BE and EAC have been carried out. In fact, our current view of the disease etiopathogenesis is governed by evidence primarily from animal models. The rat model has been successful in showing stage progression and is reproducible, making it the most widely used. The rat model has provided answers to several basic questions. However, further molecular exploration needs a more translatable model which is amenable to genetic alteration for mechanistic studies. This paper critically reviews the main surgical and genetic animal models of BE and EAC reported in the literature, emphasizing on the more recent mice models and on generating directives for future animal model studies in this field.

The Ideal Animal Model

Attwood et al.[6] reported three essential criteria for an ideal model: (i) genetic relevance to man, (ii) a conserved anatomical GEJ appropriate to man, and (iii) a naturally occurring pathophysiological GERD.

Two additional considerations—molecular validation and practical feasibility also require attention. Validation of similarity in pathogenic progression at the molecular level is

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DOI: 10.1111/cts.12304

	Human	Rodents	Dog	Pig	Primates (baboons)
Esophageal epithelium	NK	K	NK	NK	NK
Esophageal submucosal glands	Present	Absent	Present	Present	Present
Squamocolumnar transition at GEJ	Yes	No	Yes	Yes	Yes
Natural reflux	Yes	No	Yes	Yes	Yes
Natural BE-EAC	Yes	No	No	No	No†
Current laboratory success	N/A	RE-BE-EAC*	RE-BE	No	No
Suitability for lab use	No	Yes	Resource intensive	Resource intensive	No
Tumor type	EAC	EAC/ESCC	EAC	–	–
Time for progression	>10 years	Approximately 3 months	Approximately 3 years	–	–

*The developed cancerous lesions had mixed adenomatous and squamous characteristics in many experiments. †Baboons develop BE naturally but do not develop EAC.
BE = Barrett's esophagus; EAC = esophageal adenocarcinoma; ESCC = esophageal squamous cell cancer; K = keratinized; NK = nonkeratinized; RE = reflux esophagitis.
Color code: blue = comparative features in humans, white = features of established animal models, and yellow = potential animal models of BE-EAC.

Table 1. Features of current and potential animal models of BE-EAC.

crucial for translation to clinical medicine. The importance of practical feasibility of the model cannot be underestimated. The progression should be predictable and their body dimensions and lifespans be sufficient for following these animals serially with tissue specimens to detect the serial molecular changes of progression.

Current Animal Models

A wide array of animals have been utilized including rat, mice, dogs, cats, rabbits, guinea pigs, possums, ferrets, zebrafish, and baboons. The current review will focus mainly on the rodent, canine, and primate models. Rats have been at the forefront due to the convenience of laboratory handling and successful development of human like BE to EAC progression demonstrated in them by early investigators. The rat model has allowed us to develop various surgical approaches for reflux simulation and has helped in answering several fundamental pathophysiologic questions. However, the structural and physiological differences between rat and human esophagus has been a major concern in translating these results. The second most frequently used model after rodents, is the canine model. It was the first surgical reflux model but its popularity has considerably declined. While structural similarity is essential, another important aspect to consider is the evolutionary relationships between humans and these animals which in turn influences the degree of semblance in molecular aspects of basic physiology.

In general, the larger animals like dogs have the advantages of identical esophageal structure and physiology but take extremely long time to progress through the metaplasia-dysplasia-carcinoma sequence while the smaller animals like rodents are genetically relevant and alterable for mechanistic studies but have significant differences in their foregut structure. Interestingly, baboons have maximum genomic and structural homology with humans, and are the only animals known to have natural and spontaneous reflux with BE development. However, the baboon model poses various practical challenges for laboratory use and EAC development has never been reported even with chronic life-long reflux. Various animals are summarized in *Table 1* with their relative advantages and disadvantages with regards to simulating human BE.

Complete characterization of the mice genome in 2005, has provided us an exciting opportunity to develop genetic models with or without surgical facilitation to evaluate the molecular mechanism of EAC development. Many mice models have been investigated in the last decade and have achieved relatively good success.

Thus at present, there are three basic types of animal models—natural, surgical, and genetic models. Natural model: Baboons are the obligate refluxers with near uniform development of BE. However, they have not been shown to progress further to EAC. Surgical manipulation is required in almost all other models to be able to develop reflux and only rats have a documented reliability of pathogenic progression after surgical manipulation. Recently, mice models which develop metaplasia with only genetic manipulation have been created. Both genetic and environmental factors have significant contribution in the pathogenesis of EAC in humans. Thus, mice models with components of both genetic and surgical alteration have also been designed, in order to generate a pathophysiologically closer model.

Overall, we found that studies on larger animals have been much fewer as compared to rodent studies. Also, we found a trending inclination in the literature toward rodent models presumably due to their experimental convenience and documented efficacy. It must be realized that biological relevance cannot be traded with convenience. Even if we gain any meaningful insight from the mice model, we would be required to substantiate the same in a more appropriate model to establish its true significance. None of the current animal models can be considered ideal and practical.

Surgical Approaches Used

With the rarity of spontaneous reflux in laboratory animals, various surgical approaches have been applied to divert the gastric and duodeno-pancreatic secretions to the esophagus. Surgical models can be broadly divided into uncontrolled-reflux and controlled-reflux models based on the surgical approach used. Further, the uncontrolled models can be subdivided into only gastric reflux (GER), only duodenal reflux (DER), duodeno-gastric reflux with bile predominance (DgER), and duodeno-gastric reflux with acid predominance (dGER). The models

Surgical approach	Refluxate type	Animals	Success of model (at present)	Remarks	Ref.
Pyloric ligation	GER	Rats	RE	First model Acute and severe reflux	7
Wendel esophagogastroplasty ± mucosal resection	dGER	Dogs	RE-BE	Closely mimics human reflux	75
Esophago-gastro-duodenal anastomosis (EGDA)	DGER	Rats	RE-BE-EAC*	Acidic contents	26–31
Esophago-duodenal anastomosis (EDA)–side-to-side and end-to-side	DGER	Rats, mice	RE-BE-EAC*	Highly alkaline reflux	23
Esophago-duodenal anastomosis with gastrectomy (EDA + G)	DER	Rats, mice	RE-BE-EAC*	Only alkaline reflux	76,77
Esophago-jejunostomy (EJ)	DGER	Rats, mice	RE-BE-EAC*	Highly alkaline reflux	9,35,42,51,52
Esophago-jejunostomy with gastrectomy (EJ + G)	DER	Rats, mice, dogs	RE-BE-EAC*	Only alkaline reflux	35
External esophageal perfusion (EEP)	Controlled	rats, mice, dogs	RE	Controlled reflux models	32–34

*The developed cancerous lesions have mixed adenomatous and squamous characteristics in many experiments.

GER = gastroesophageal reflux; DGER = duodeno-gastro-esophageal reflux; dGER = gastric predominant duodeno-gastro-esophageal reflux; RE = reflux esophagitis; BE = Barrett's esophagus; EAC = esophageal adenocarcinoma; LES = lower esophageal sphincter; EGDA = esophago-gastro-duodenal anastomosis; EDA = esophago-duodenal anastomosis; EDA + G = esophago-duodenal anastomosis with gastrectomy; EJ = esophago-jejunostomy; EJ + G = esophago-jejunostomy with gastrectomy; EEP: exogenous esophageal perfusion.

Table 2. Various surgical approaches used to create animal reflux models.

utilizing these approaches have been tabulated to display the spectrum of techniques employed (*Table 2*)

Initial uncontrolled models

Selye[7] created the first animal reflux model by ligation of the gastric pylorus in rats. This model although successful in inducing reflux, was criticized as it lead to acute and massive acidic reflux which was very dissimilar to human reflux disease. Later, Omura[8] created a chronic gastric outlet obstruction model to create chronic reflux in rats. These attempts failed at producing BE or EAC, only leading to reflux esophagitis.

In 1989, Pera et al.[9] reported that they induced EAC successfully in rats by creating an esophago-jejunostomy (EJ) anastomosis with subsequent exposure to nitroso-amines as exogenous carcinogens. Later more and more complex surgical approaches were used to separately evaluate the contribution of various refluxate components in the disease pathogenesis. Levrat et al. first endeavored to design multiple surgical approaches in rats to simulate human reflux. Even today, Levrat's models with minor modifications are being used successfully. The most successful and validated models have been the EDA (esophago-duodenal anastomosis) and esophago-gasto-duodenal anastomosis (EGDA) models. With regards to duodeno-esophageal reflux (DER), EJ model produces more bile reflux than EDA and EGDA models. Attempts at developing only DER in animals through gastrectomy has often been met with greater postoperative morbidity and mortality.[10,11] A more practicable approach used for separately evaluating the toxicity of alkaline components is to simply suppress acid pharmacologically with EDA or EGDA.[12]

Studies were also designed to see if exogenous exposure to carcinogens,[9,13–21] including nitrosamine compounds, iron,[22–24] or fat[16,25] could help promote carcinogenesis. They consistently found exogenous carcinogen exposure promoted squamous cell cancer development.[9,13–15,18–20] An exception is iron supplementation which favored EAC formation.[22–24] Clark et al. and Chen et al. demonstrated that addition of fat in the diet played an important role in adenocarcinogenesis.[16,25] In the current EDA and EGDA models,[26–31] BE developed in 3–77% cases within a time period ranging from 20 to 70 weeks while the incidence of EAC reported was 7–83%, requiring 30–70 weeks for development.

These surgical models that divert the gastroduodenal contents to the esophagus do not have control over the amount and concentration of the refluxate. Although some approaches allow predominance of one component over the other, it is difficult to completely separate the individual component of the refluxate. Moreover, postoperative malnutrition and stress deters any fruitful follow-up in these animals. Rodents, particularly mice cannot withstand excessive surgical stress and most die within the immediate postoperative period.

Controlled reflux models

Cross et al.,[32] in 1951, used an external esophageal perfusion approach with bile and/or pancreatic secretions for the first time and successfully developed esophagitis. These results were confirmed by Redo,[33] while conducting similar canine experiments. Recently Li et al.,[34] in an intention to study the effect of pure acid and pure bile induced esophageal injury, utilized a novel exogenous esophageal perfusion model. In their experiment, three groups of rats underwent upper esophageal cannulation to deliver saline, bovine bile, or hydrochloric acid for 7 days at a rate of 10 μL/hour through subcutaneously implanted osmotic pump. They showed that both bile and acid perfusion dramatically elevated oxidative damage, increased cell proliferation, and apoptosis. This quasisurgical approach has many advantages including control over the concentration and components of refluxate, ease of the procedure and considerably lesser morbidity.

Rat Models

Several strains of rats, e.g., Sprague-Dawley, F344, Wistar, have been used to generate reflux models. Experiments with rats date back to 1962 by Levrat et al.[35] when his team used different surgical approaches to create reflux in order to evaluate the contribution of various reflux components. Esophagitis was observed within a month in all the rats with duodenal and pancreaticobiliary refluxates with/without gastric refluxates. No esophagitis was observed with acid only refluxers (esophagus anastomosed with prepyloric stomach), with gastroduodenal only refluxers (EJ with pancreaticobiliary diversion) or duodenal only refluxers (EJ with pancreaticobiliary diversion and gastrectomy). This highlighted the relative importance of alkaline components. This study set the standards for different surgical options to be used in future models. Chen et al.[24] followed the EGDA rat model through stages of GERD, multi-layered epithelium (MLE), CLE, dysplasia, and EAC. They showed that BE and MLE in rats resembled human BE and MLE in its morphology, mucin features, and expression of differentiation markers. As the EGDA model became more popular, there was speculation that columnar cells in the esophagus were derived through cell migration from the jejunum. It is through selective expression profiles of Trefoil factor genes (TFF-1, TFF-2, TFF-3) in the esophagus, that the intestinal metaplasia produced was confirmed to be intrinsic to the esophagus.[36]

There has been lot of concern regarding the translatability of these studies to human disease. As shown in *Table 1*, rats differ in basic homology with human esophagus. Recently, Horn et al.[37] demonstrated that rodents lack a vomiting (emetic) reflex in contrast to other mammals. They speculated it to be due to a relatively long abdominal esophagus, reduced muscularity of the diaphragm and/or absent brainstem neurological component. The reproducibility of the rat model has been quite unpredictable with unexpected development of mixed tumor types, both adeno and squamous tumors.[38] The adenocarcinomas which developed were mucinous in characteristics and not glandular in most cases. Buskens et al.[39] compared them to "esophagitis cystica profunda" which are reactive mucous producing lesions histologically resembling adenocarcinomas. The absence of deep invasion and metastasis also lead to questions whether the induced malignancies differ in their aggressiveness from human disease. Yano et al.[40] showed that even rats of the same species and gender differ with respect to their behavioral and growth patterns between different continents. The American Wistar male rats gained weight much faster and had a significantly lower 3-week survival as compared to the Japanese counterparts even with same environmental and feeding conditions. This further complicates reproducing and extrapolating from the already published results. With regards to follow-up, endoscopic assessment is erratic due to extraluminal development of majority of tumors and low yield from endocopic biopsies in rats. Recently, Kosovec et al.[41] demonstrated the usefulness and advocated the use of MRI rather than endoscopy for tumor assessment during follow-up. In conclusion, there is a need for a better model to overcome these concerns.

Mice Models

Mice models are well-suited for molecular studies given that their genome is completely sequenced and transgenic mice are commercially available today.[42] There have been many attempts to utilize genetically modified[41–46] and xenograft mice models[47–49] in BE research. However, creation of reflux in mice models often leads to many dying in the early postoperative period due to difficulty in performing surgery at the scale of their sizes and their intolerance to surgical stress. Furthermore, mouse reflux models yield much lower incidence of BE as compared to rat reflux models even with the addition exogenous carcinogens. Novel surgical approaches like recently published by Davelaar et al.[50] have the potential to overcome this impediment and are described in detail below. We have divided the mouse models below, based on the approach used.

Purely surgical models

Xu and associates[51] in 2001 pioneered in creating mice reflux models for the study of EAC pathogenesis. They divided 108 Swiss Webster mice into three experimental groups; EJ alone, EJ with carcinogen, and carcinogen alone and found BE incidence to be 42.4%, 20%, and 12.5% while EAC developed in 6.1%, 37.1%, and 12.5% of these mice, respectively. They showed no mortality in their 19-week follow-up. Their study demonstrated that success with mice models can be at par with the well-established rat models. Another study that very clearly demonstrated the viability of purely surgical mice reflux models is by Raggi et al. in 2010. They showed a 60% incidence of BE at 16 weeks after EJ in forty BALB-c mice, and a 55% incidence of EAC by 20 weeks. They reported an overall mortality of 17%.

Pham et al.[42] in 2013 performed microsurgical EJ anastomosis in 20 C57Bl/6 mice on similar lines to Xu et al. They demonstrated a mere 7% BE incidence by 52 weeks confirmed both by histologic and immunohistochemical analysis. The overall mortality was 30%. This study was undertaken to check the practical feasibility of using the mouse model before subjecting the mice for genetic manipulation studies. Thus they used C57Bl/6 mice strain which has its genome most well characterized and is the strain most employed for creation of knockout and knock-in mouse. They suggested that the difference in mice strains may play a substantial role in the observed differences in susceptibility for BE and EAC development. In this context, Babu et al. compared the incidence of early mucosal changes due to reflux injury between the two most common mouse models, C57Bl/6 and BALB-c using a side-to-side EDGA approach. They found the C57Bl/6 to be immune to early mucosal injury by reflux probably due to the inherent disruption of the gene for group IIa secretory phospholipase A2 in these mice.

While previous studies used end-to-side EJ approach, Aikou et al.[52] in 2013 in their study to evaluate contribution of bone marrow stem cells in the origin of BE, used an side-to-side EJ approach for the first time in mice. Encouragingly, BE was observed in 28% and 41% of C57BL/6 mice at 20 and 40 weeks respectively after surgery. An overall mortality of only 4% was achieved. However, only a single case of ESCC development was noted and none of the mice developed EAC.

Davelaar et al.[50] recently developed a novel suture-less method to create a side-to-side EJ model by implantation of neodymium micromagnets in both esophagus and jejunum which then oppose to fistulate within days by pressure necrosis. This approach causes significantly lesser morbidity and mortality in mice. At 9 week landmark, 70% of the mice developed RE while BE developed in half the mice.

Genetic models with surgical facilitation

Genetically altered mice could provide knowledge on the role of a particular gene in BE induction and progression to EAC when rates of these events are compared between altered and unaltered

groups. This could reveal, although piecemeal, the molecular disruption which occurs during neoplastic progression.

Fein et al.[43] in 2001 reported first such model. They studied the effect of P53 gene knockout (P53KO) in a surgical mouse reflux model utilizing EJ with gastrectomy. Although P53KO mice showed higher rate of EAC development, only four out of 12 mice survived after 24 weeks, just two developed EAC and one ESCC. In another recent study using a larger sample size, 28 of 32 operated P53KO mice died within 20 weeks.

Hao et al.[46] studied the effects of P53 and P16 mutations on A/J mice reflux models. They found none of the mice to develop either typical BE or EAC. Instead they observed scatted mucinous cells, unlike BE found in rat models and ESCC developed in these mice. Their study also suggests a possible influence of mice strain on response to reflux mucosal injury.

Another KO surgical model used in two studies is the P27 knockout (P27KO) model. Ellis et al. found that with P27KO, the incidence of BE and EAC was 26% and 23.3%, respectively and was significantly higher than controls. Surprisingly, there was no mortality reported in their EJ± carcinogen model at 20-week follow-up. Lechpammer et al.[45] using the same model as Ellis et al., showed that flavopiridol, a cyclin-dependent kinase inhibitor could reduce the prevalence of BE and EAC in p27 knockout mice by almost two-thirds, further highlighting the importance of P27 gene in EAC pathogenesis.

Purely genetic models

These models are intriguing in the aspect that they are able to produce BE like metaplastic changes only with the help of genetic alteration. However, they are created by single gene insertion or deletion in all cells of the whole body or a whole organ, which is a rather oversimplification of the process of clonal evolution naturally occurring in the carcinogenic field. Additionally, many of these mutations used for their creation are lethal.

Crawford et al.[53] first reported BE development by genetic manipulations alone in mice. They reported occasional development of BE mice defective for thrombospondin-1 but no development of EAC was found. The Sonic hedgehog transgenic mice model developed by Wang et al. demonstrated Hh pathway activation to be sufficient for expression of columnar cell characteristics and BE development in Swiss Webster mice.[54] Another mouse model developed by McKeon et. al.[55] of p63-deficient neonatal mice exhibited a BE like columnar epithelium with positive staining with alcian blue and periodic acid-Schiff. However, this model also showed no progression to EAC. Quante et al.[56] developed an innovative approach to create a genetic mouse model of inflammation-dependent esophageal metaplasia without surgical intervention. They overexpressed IL-1β in the esophageal and squamous forestomach mucosa of mice by transfecting with an EBV-L2-IL-1β transgene. The mice exhibited esophagitis and progressed to BE by 12 months and then spontaneously developed EAC with time. On addition of bile acids to the drinking water (0.2% deoxycholic acid), accelerated the onset of BE and EAC. Furthermore, combined addition of bile acids and nitrosamine markedly accelerated BE and EAC.

Canine Model

Canine models were one of the initially used higher animal models of BE. Although dogs have the advantage of structural similarity with the human esophagus, interest in this model gradually declined due to difficult laboratory handling and more importantly, long time taken for the progression to occur. They develop BE from 1 year upto 3 years with[57] or without mucosectomy.[58] The development of EAC can take upto 5 years. The report by Redo et al.[33] in 1959 marks the first attempt at using dogs in an external perfusion model. In 1970, Bremner et al.[57] developed the first surgical canine model of BE. They used surgical and pharmacological gastric acid augmentation along with mucosectomy to expedite the development of BE. An important land mark in BE research was the canine study by Gillen et al.[59] where they demonstrated BE to develop even when the esophageal mucosal defect was separated by normal squamous mucosa disproving the gastric migration hypothesis. In another similar canine study by Li et al.[60] in 1994, the use of acid suppression caused partial columnar regeneration with squamous islands. They proposed that the degree and depth of injury is critical determinant of extent of metaplastic change. Severe injury destroys both mucosal and glandular cells, regeneration would be homogeneously columnar. While when the injury is less severe, both cells will proliferate but columnar will predominate because of its rapid turnover. Narbona et al.[58] attempted and developed BE in dogs without mucosectomy and acid augmentation, which corresponds more to the natural course of BE in the humans. They were able to produce BE within a year with changes occurring earlier in the group induced with mixed reflux as compared to only acid reflux. The most recent canine model experiment is from Kawaura et al.[61] in 2001. EAC developed in one animal from each of the groups of 26 pure acid and 24 alkaline refluxers at around 5 years.

Baboons

Reports on baboons for the study of BE pathogenesis are very recent.[62] They have attracted great scientific interest as they have been found to naturally have reflux since birth. The esophageal structure and gastric acid pH are similar in both baboons and humans[63–66] but their oblique body posture makes the reflux more continuous unlike humans.[67] Baboons who died from natural causes were examined by Rubio et al. for the presence of BE and histological analysis revealed all animals to have developed BE ranging from as short as 1 mm to as long as 40.2 mm. However, there are no reports of EAC development in baboons in the literature. Interestingly, Rubio et al. demonstrated that BE in baboons has numerous "sialomucin-overstuffed cells" which is rarely reported in humans. This model has been used in a very few studies but has a lot of potential for future BE research. Although, primate research is highly effort intensive, their natural history of BE and evolutionary closeness with humans makes them special. They could be used to validate findings found in lower animals before beginning human trials.

Potential Future Models

Among other large animals, pigs have been extensively utilized in biomedical research. No successful swine BE model has been reported so far.

Pigs

Miniaturized pigs have become increasingly more used in the laboratory due to their docile temperament and ease of handling. The anatomy, physiology, and pharmacology of the esophagus and gastroesophageal junction in pigs has been shown to be similar to humans.[68,69] Unlike rodents, their body size is convenient for surgical and endoscopic interventions. Additionally, they can

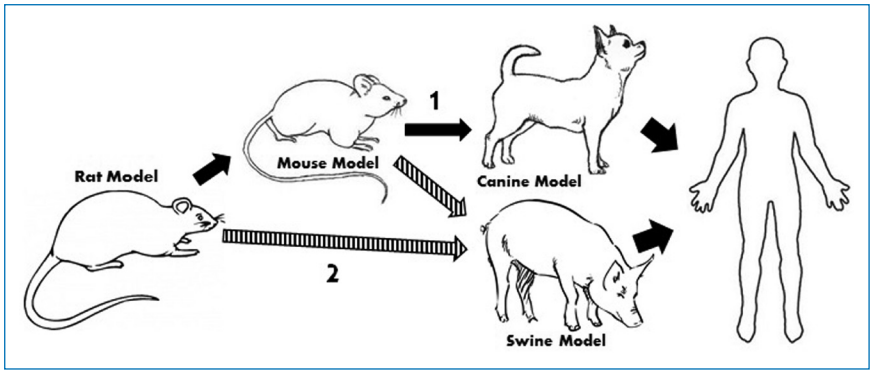

Figure 1. Approach for future translation of animal model research on BE-EAC. Approach 1: Findings from rat studies can be tested in mouse models for mechanistic insight and then tested in more physiologic canine or swine models for preclinical validation before human studies. Approach 2: Findings from rat studies can directly be tested in swine models both for molecular and physiologic validation before human translation.

be bred in controlled environments and are more amenable to restraint than other larger animals.

Studies looking at pig esophagus are scarce and scattered in the literature. Cristie et al.[70] studied the distribution and relative catalytic activities of five plasma membrane associated enzymes in the esophageal mucosa and found it to be similar to humans. Abdulnour et al.[71] showed similar cytokeratin and lectin staining in pig submucosal glands to the staining pattern in human BE. Continuous ambulatory pH monitoring was done by implanting a pH sensitive radiotelemetry capsule to the esophageal wall in pigs and spontaneous reflux was found to occur.[72] However, there was no endoscopic or histologic evidence of esophagitis or metaplasia. To date, no successful swine BE model reported yet. Considering the fact that pigs are evolutionarily close to humans[73] and that transgenesis in pigs is now possible,[74] swine BE model can bridge the gap between primates and rodents in terms of translatability and suitability for lab studies.

Looking Ahead

Much progress has been made with surgical animal models of BE and EAC in small animals including rats and mice. Mice models need improvisation of reproducibility of EAC development and can then be greatly instrumental in molecular studies in the future. Newer less invasive surgical approaches to develop mice reflux models may further facilitate molecular studies. However, structural and functional differences between human and rodent need cautious consideration. Learning from the knowledge acquired from the rat/ mice models, BE models in higher animals should be endeavored. Transgenic modification in higher animal models has been shown to be realizable and ushers great hope for molecular studies in higher models. This two-step approach may allow safer translation of research findings (*Figure 1*).

Acknowledgments

Research reported in this publication was supported by the LB692 grant to TL and SKM and National Institutes of Health research grants to DKA (R01HL104516, R01HL116042).

Disclosure

All authors have read the journals publication policy and have no conflict of interest to disclose.

Author Contributions

HK and KRL contributed by reviewing literature and drafting the manuscript, THL and DKA were instrumental in critical revision of the manuscript and SKM made substantial contribution to the design of the manuscript and took overall responsibility of the project.

Grant Support

NIH (DKA; R01HL104516 and R01HL116042) and TL, SKM (LB692).

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